The 1.8-Å Crystal Structure of InaD PDZ1 Complexed with its Peptide Target Reveals a New Mode of PDZ Domain Binding

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Drosophila phototransduction is a model system for the study of G-protein coupled phospholipase-C (PLC) signaling pathways in complex organisms. In this cascade light activates the seven-transmembrane receptor rhodopsin, which in turn activates G_q, allowing its dissociation into signaling-competent α and $\beta\gamma$ subunits. $G_q\alpha$ induces the PLC-β4 homolog no receptor potential A (norpA) to cleave phosphatidylinositol-4,5-bisphosphate (PIP_2) to the second messengers inositol tri-phosphate (IP_3) and diacylglycerol (DAG), leading to the transient opening of cation channels and depolarization of the photoreceptor cells. The light response is deactivated by phosphorylation by protein kinase C (PKC).

Proper targeting of norpA, among other phototransduction proteins, to the photoreceptor cells requires the multi-domain scaffolding protein inactivation no after-potential D (inaD). InaD contains five tandem PDZ protein interaction domains, each of which can bind one or multiple phototransduction proteins. PDZ domains bind to the extreme carboxy-terminal (C-terminal) three amino acids of their targets, including the free carboxyl group. InaD PDZ1 binds to norpA, whose C-terminal sequence is FCA. Crystals of PDZ1 bound to a norpA C-terminal heptapeptide were grown, and multiwavelength anomalous diffraction (MAD) data was collected at NSLS beamline X4A from both selenomethionine- and mercury-labeled samples. While heavy atom peaks were found in both datasets, the resulting phases alone were not enough to solve the crystal structure. The structure of the PDZ1/heptapeptide complex was determined by molecular replacement to 1.8-Å resolution, with an R-factor of 22% and a free R-factor of 23%. This high-resolution structure reveals a novel mode of PDZ domain binding—the formation of an intermolecular disulfide bond. *In vitro* protein binding studies show PDZ1/norpA formation can be abolished with addition reductant, suggesting that the disulfide bond is physiologically relevant. Precedent for intracellular disulfide bond formation includes homotetramerization of PSD-95 in post-synaptic densities. These results suggest that some peripheral membranes proteins, including PSD-95, its homologs, and certain PDZ/protein complexes may be bound in semi-permanent signaling scaffolds.